JOURNAL OF VIROLOGY, Mar. 1984, p. 741–747 Vol. 49, No. 3
0022-538X/84/030741-07$02.00/0


# Mapping of the Structural Gene for the Herpes Simplex Virus Type 2 Counterpart of Herpes Simplex Virus Type 1 Glycoprotein C and Identification of a Type 2 Mutant Which Does Not Express This Glycoprotein

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Received 31 August 1983/Accepted 7 November 1983

The gene encoding glycoprotein F (gF) of herpes simplex virus type 2 (HSV-2) was mapped to the region of the viral genome from 0.62 to 0.64 map units. This region is colinear with, and partially homologous to, the region of the HSV-1 genome previously shown to encode gC. Mapping of the gF gene was done by insertion of HSV-2 DNA fragments into the thymidine kinase gene of an HSV-1 virus and screening of the resultant recombinant viruses for the expression of gF. In this way, DNA sequences necessary for the expression of gF in infected cells were also delimited. Because several plaque morphology mutants (syncytial mutants) of HSV-1 have previously been shown to be gC$^-$, a syncytial mutant of HSV-2 ($G_P$) was tested for the expression of gF. It was found to be gF$^-$, indicating that gF is not essential for replication of HSV-2 in cell culture, just as gC is not essential for replication of HSV-1. This result also suggests that the gF$^-$ and gC$^-$ phenotypes are related in the same, as yet undefined, way to the expression of a syncytial marker. A proposal to change the name of HSV-2 gF to gC (gC-2) is discussed.

Herpes simplex virus types 1 and 2 (HSV-1 and HSV-2) specify several glycoproteins that are expressed on both viral and infected cell surfaces. The HSV-1 glycoproteins have been designated gB, gC, gD, and gE (31). For HSV-1 gB, gD, and gE, related counterparts of similar electrophoretic mobility have been identified in HSV-2 (6, 8, 20, 21, 23–25, 29) and have been assigned the same alphabetic designations. Until recently HSV-1 gC was thought to be type specific. Evidence has recently emerged, however, indicating that gF (1, 2) is the HSV-2 counterpart of HSV-1 gC. We have reported mapping results (22) that suggested that the gene for HSV-2 gF could be colinear with the gene for HSV-1 gC. Zweig et al. (37) described a monoclonal antibody to gF that cross-reacts with gC, and we have demonstrated that a rabbit antiserum prepared against HSV-1 membrane proteins contains anti-gC antibodies that cross-react with gF (36).

Here we present more refined mapping results that show that the gene for HSV-2 gF is located within a 2.9-kilobase (kb) region of the HSV-2 genome (0.62 to 0.64 map units) that is colinear with the gene for gC (0.63 to 0.64 map units [9, 14]) in HSV-1. This mapping was performed by insertion of appropriate HSV-2 DNA fragments into the thymidine kinase (TK) gene of the gC$^-$ strain HSV-1(MP) and demonstration that insertion of particular fragments correlated with expression of gF by the recombinant viruses. Mapping of the gene for gF in this way also defined boundaries of sequences necessary for the expression of gF in infected cells.

Although the functions of HSV-1 gC and HSV-2 gF are not known, spontaneous HSV-1 gC$^-$ mutants have emerged in cell culture (4, 11, 12), suggesting that gC is not necessary for viral replication in such cell systems. These spontaneous HSV-1 gC$^-$ mutants were originally recognized because of their syncytium-forming (*syn*) phenotype. Although it has been shown that the absence of gC-1 expression is not directly responsible for the *syn* phenotype (13, 14), spontaneous emergence of the mutant phenotypes (gC$^-$ and *syn*) is often coincident, suggesting some as yet undefined relationship between the two. Additional analogies between HSV-1 gC and HSV-2 gF are provided here by the identification of an HSV-2 gF$^-$ mutant in which the gF$^-$ phenotype is found in association with a syncytial phenotype. Therefore, neither gC nor gF is essential for viral replication in cell culture, and spontaneous emergence of both the gF$^-$ and gC$^-$ mutant phenotypes can be coincident with the expression of a *syn* marker.

## MATERIALS AND METHODS

**Cells and viruses.** HEp-2 (human epidermoid carcinoma-2) cells and African green monkey kidney (Vero) cells obtained from the American Type Culture Collection were used in these studies. The cells were grown as monolayer cultures in Dulbecco modified Eagle minimal essential medium supplemented with 10% fetal bovine serum. Viruses used were the gC$^-$ strain HSV-1(MP) (12) and HSV-2 strains 333 (7; obtained from F. Rapp, Hershey Medical Center, Hershey, Pa.) and $G_P$ (4). The recombinant viruses produced in these studies are described below.

**Plasmids.** pRB103 [26; *Bam*HI fragment Q from HSV-1[F] DNA cloned into pBR322] was provided by B. Roizman. Plasmids constructed for the generation of thymidine arabinoside-resistant (AraT$^r$) insertion mutant viruses were derived from pRB103 (Fig. 1). For pKZ669 and pKZ672, the 4.9-kb *Sac*I fragment of HSV-2 DNA, as indicated, was ligated in different orientations into the single *Sac*I site of pRB103, interrupting the coding sequence of the HSV-1 TK gene (17, 32). For pKZ801 and pKZ802 the 2.9-kb *Sal*I fragment of HSV-2 DNA was inserted in different orientations into the single *Bgl*II site of pRB103 by blunt end ligation after an end-filling reaction with the Klenow fragment of *Escherichia coli* DNA polymerase I. *Bgl*II sites were regenerated by this ligation, except for one *Bgl*II site in pKZ801, which was lost (Fig. 1). These insertions interrupt the TK gene between the site for the initiation of transcrip-

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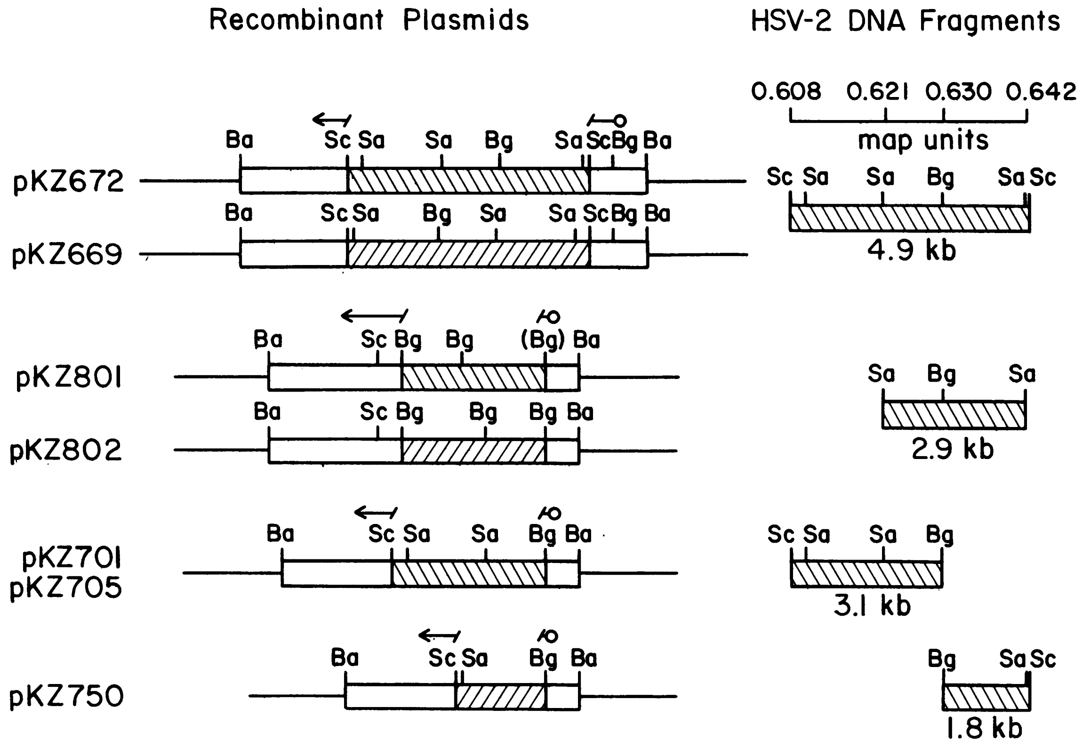


FIG. 1. Recombinant plasmids derived from pRB103 [*Bam*HI-Q fragment of HSV-1(F) DNA inserted into pBR322] and HSV-2 DNA fragments used in their construction. HSV-2(333) DNA fragments were inserted into sites within the HSV-1 TK gene located in the *Bam*HI-Q fragment of pRB103. HSV-1 *Bam*HI-Q sequences are indicated by open boxes, and HSV-2 DNA sequences are indicated by striped boxes, the direction of the stripe denoting their orientation. pBR322 sequences (not drawn to scale) are indicated by lines. Sequences homologous to uninterrupted TK mRNA, along with the direction of transcription (17, 32), are indicated by the broken arrows. The locations of the HSV-2 DNA fragments within the HSV-2 genome are indicated on the right. Map units were calculated by using the values of 0.580 and 0.630 for the left and right boundaries, respectively, of the HSV-2 *Bgl*II-N DNA fragment. Abbreviations: Bg, *Bgl*II; Sa, *Sal*I; Ba, *Bam*HI; Sc, *Sac*I.

tion and the coding sequence (17, 32). pKZ701 and pKZ705 are presumably identical plasmids constructed by ligation of the *Sac*I-*Bgl*II 3.1-kb HSV-2 DNA fragment between the *Sac*I and *Bgl*II sites of pRB103, resulting in the loss of 500 base pairs of the TK gene. pKZ750, containing the 1.8-kb *Bgl*II-*Sac*I fragment of HSV-2 DNA, was also constructed by ligation of this fragment between the *Bgl*II and *Sac*I sites of pRB103.

**Preparation of viral DNA.** HSV-1(MP) viral DNA was isolated from infected Vero cells by a procedure (3) adapted from that described by Walboomers and ter Schegget (33) with additional modifications. Briefly, infected cells were washed with phosphate-buffered saline, suspended in 0.01 M Tris-hydrochloride buffer (pH 7.6) containing 0.01 M EDTA and 1% Nonidet P-40, held on ice for 10 min, and centrifuged at low speed to pellet the nuclei. To the cytoplasmic fraction, sodium dodecyl sulfate was added to 0.6%, and pronase was added to 1 mg/ml, followed by incubation at 37°C for 4 h and centrifugation to equilibrium on NaI density gradients containing ethidium bromide. The single viral DNA band was visualized by exposure to UV light and, after recovery, was dialyzed overnight against TE buffer (0.01 M Tris-hydrochloride buffer [pH 7.6], 0.001 M EDTA), extracted with isoamyl alcohol, and dialyzed exhaustively against TE buffer. Viral DNA from AraT$^r$ recombinant viruses produced in these studies was prepared as above, except that after pronase treatment, the DNA was successively extracted with phenol, phenol-chloroform-isoamyl alcohol (50:48:2), and chloroform-isoamyl alcohol (24:1) and then ethanol precipitated.

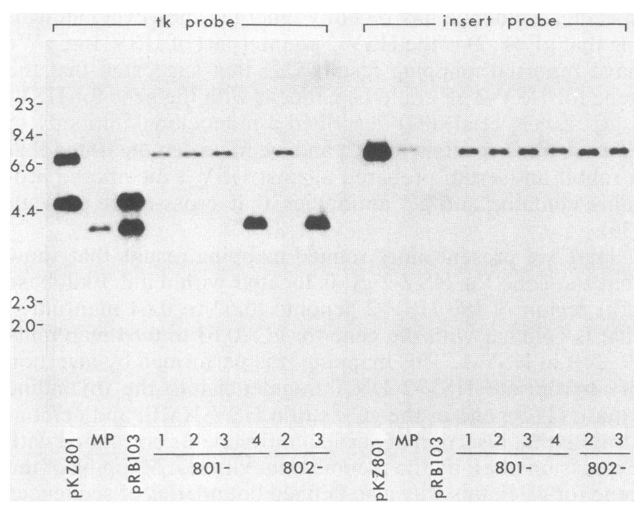


FIG. 2. Southern blot analysis of genomic DNAs from AraT$^r$ viruses that were digested with *Bam*HI and hybridized to $^{32}$P-labeled pRB103 DNA (TK probe) or to the $^{32}$P-labeled 2.9-kb HSV-2 *Sal*I DNA fragment (0.621 to 0.641 map units) purified from agarose gels (insert probe). The TK probe labeled pBR322 sequences, as well as *Bam*HI Q fragment sequences from HSV-1 DNA, whereas the insert probe labeled only the HSV-2 DNA sequences indicated. The AraT$^r$ viral isolates were from transfections with either pKZ801 or pKZ802 DNA and MP DNA. *Bam*HI-digested DNAs from HSV-1(MP), pKZ801, and pRB103 are also shown. The molecular weight markers on the left, shown in kb, are from an *Hin*dIII digest of λ DNA.

**Cloning procedures, purification of plasmid DNA, and purification of restricted DNA fragments from agarose.** The methods used for the construction and screening of cloned DNA fragments and for the isolation of plasmid DNA by centrifugation to equilibrium in cesium chloride gradients were as described by Post et al. (26). In addition, ligation of restricted DNA fragments with noncomplementary, protruding 5′ ends by blunt-end ligation after an end-filling reaction with the Klenow fragment of *E. coli* polymerase I was as described by Wartell and Reznikoff (34). Digestion of DNA with restriction enzymes was as specified by the manufacturer (New England Biolabs). Purification of restricted DNA fragments from agarose was by electrophoresis of the DNA through SeaPlaque (FMC Corp., Rockland, Maine) agarose, melting of the gel slices at 66°C, addition of NaCl to 0.1 M, phenol extraction, and dialysis against TE buffer.

**Southern transfer of DNA and hybridization.** Viral or plasmid DNA (or both) was digested with appropriate restriction enzymes, electrophoresed on 0.75% agarose gels, and transferred to nitrocellulose by the procedure of Southern (30). Hybridizations were done at 65°C in 6× SSC (0.9 M NaCl, 0.09 M sodium citrate), 5× Denhardt solution (0.1% Ficoll, 0.1% polyvinylpyrrolidone, 0.1% bovine serum albumin), 0.5% sodium dodecyl sulfate, 50 μg of calf thymus DNA per ml, 30% formamide, and the denatured $^{32}$P-labeled DNA probe. After hybridization for 12 to 24 h, nitrocellulose filters were washed as described by Maniatis et al. (15). Nick translation kits obtained from New England Nuclear Corp. (Boston, Mass.) were used to produce the $^{32}$P-labeled DNA probes.

**Construction of AraT$^r$ recombinant viruses.** Vero cells were cotransfected with mixtures of HSV-1(MP) viral DNA and the appropriate *Bam*HI-digested plasmid DNA (pKZ669, pKZ672, pKZ801, pKZ802, pKZ701, pKZ705, or pKZ750). Calcium phosphate precipitates of DNA were prepared which contained 0.25 or 0.4 μg of HSV-1(MP) DNA, 20 μg of salmon sperm DNA, and 0.05, 0.15, or 0.45 μg of plasmid DNA per 0.5 ml, and cells were treated with this DNA by the procedure of Graham and Van der Eb (10) as modified by Wigler et al. (35). Progeny virus from the transfected cells were plated on Vero cells under agarose in the presence of AraT (Raylo Chemical, Edmonton, Alberta, Canada) at 100 μg/ml (18) to select for AraT$^r$ recombinants. AraT$^r$ isolates were plaque purified twice in the presence of AraT, except for isolates 750-1 through 750-4, which were not plaque purified.

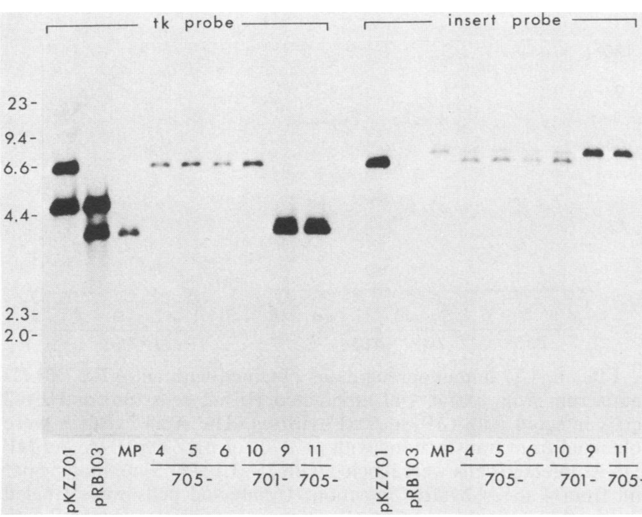


FIG. 3. Southern blot analysis of genomic DNAs from AraT$^r$ viruses that were digested with *Bam*HI and hybridized to $^{32}$P-labeled pRB103 DNA (TK probe) or to the $^{32}$P-labeled 2.9-kb HSV-2 *Sal*I DNA fragment (0.621 to 0.641 map units) purified from agarose gels (insert probe). The AraT$^r$ viral isolates were obtained from transfections with either pKZ701 or pKZ705 DNA and MP DNA. *Bam*HI-digested DNAs from HSV-1(MP), pKZ701, and pRB103 are also shown. The molecular weight markers on the left, shown in kb, are from an *Hin*dIII digest of λ DNA.

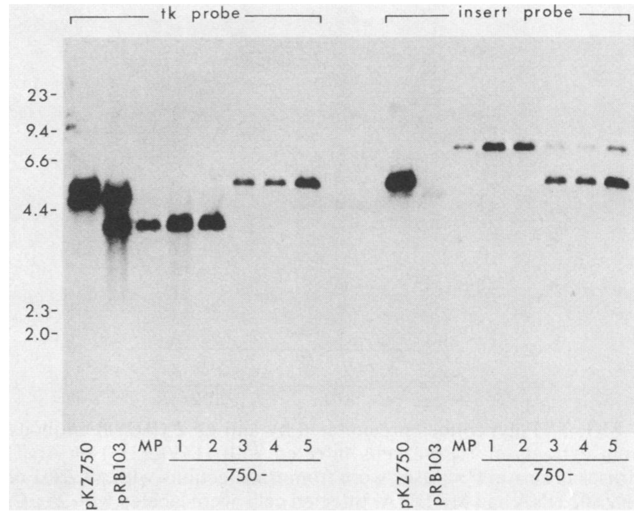


FIG. 4. Southern blot analysis of genomic DNAs from AraT$^r$ viruses that were digested with *Bam*HI and hybridized to $^{32}$P-labeled pRB103 DNA (TK probe) or to the $^{32}$P-labeled 2.9-kb HSV-2 *Sal*I DNA fragment (0.621 to 0.641 map units) purified from agarose gels (insert probe). The AraT$^r$ viral isolates were obtained from transfections with pKZ750 DNA and MP DNA. *Bam*HI-digested DNAs from HSV-1(MP), pKZ750, and pRB103 are also shown. The molecular weight markers on the left, shown in kb, are from an *Hin*dIII digests of λ DNA.

**Antibodies and immunoprecipitations.** Hybridoma cell lines secreting monoclonal antibodies directed against HSV-1 or HSV-2 glycoproteins were isolated by M. Para in our laboratory. The monoclonal antibodies used in these studies (in the form of ascites fluid) were previously described (19, 22) and are as follows: III188 directed against gF-2 (anti-gF-2), II73 directed against gC-1 (anti-gC-1), and III114 directed against gD-1 and gD-2 (anti-gD-1/2). Monoclonal antibody 151.2, specific for gC-1, was a gift from J. Glorioso and M. Levine (University of Michigan, Ann Arbor). Rabbit antiserum R#71, prepared against HSV-2 gF, was previously described (36).

Immunoprecipitations were performed as previously described (36) with extracts from infected HEp-2 cells that were labeled with [$^{35}$S]methionine from 4 to 24 h after infection, and the precipitated products were analyzed on 8.5% sodium dodecyl sulfate-polyacrylamide gels cross-linked with *N*,*N*′-diallyltartardiamide (11).

## RESULTS

**Construction of recombinant viruses.** To more precisely define the location of the gene for gF-2 in the HSV-2 genome, recombinant viruses were constructed that contained defined regions of HSV-2(333) DNA inserted into the

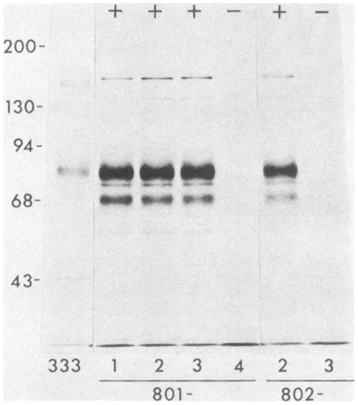


FIG. 5. Polypeptides precipitated by anti-gF-2 (III188) antibody from extracts of HEp-2 cells infected with HSV-2(333) or AraT$^r$ viruses. The AraT$^r$ viruses were from transfections with pKZ801 or pKZ802 DNA and MP DNA. Infected cells were labeled with 25 μCi of [$^{35}$S]methionine per ml from 4 to 24 h after infection. The + and − symbols show the results of Southern blot analyses performed with the DNA from each of the AraT$^r$ viruses and indicate the presence or absence, respectively, of HSV-2 DNA insertions within the TK genes of the AraT$^r$ viruses.

TK gene of the gC$^-$ strain HSV-1(MP), and these viruses were then analyzed for the expression of gF. Figure 1 shows the recombinant plasmids produced for these constructions and the map locations of the HSV-2 DNA fragments that were inserted into these plasmids. HSV-2 DNA fragments chosen for insertion were a 4.9-kb *Sac*I fragment (located between 0.608 to 0.642 map units in the genome) that hybridized to the HSV-1(F) *Sal*I R DNA fragment, within which the gene for gC-1 is located (9, 14), and subregions of this fragment.

To produce the recombinant viruses, plasmid DNAs were digested with *Bam*HI and, together with HSV-1(MP) DNA, were used to transfect Vero cells. Viral progeny were selected for loss of TK expression in the presence of AraT, and the DNAs for these viral isolates were analyzed to determine whether they had insertions in their TK genes. Fig. 2, 3, and 4 show Southern blot analyses of genomic DNAs from AraT$^r$ isolates digested with *Bam*HI and hybridized to probes for the TK gene or for the HSV-2 DNA sequences inserted into the TK gene.

Figure 2 shows that three of four AraT$^r$ isolates obtained from transfections with pKZ801 (801-1, 801-2, and 801-3) and one AraT$^r$ isolate obtained from transfection with pKZ802 (802-2) contained insertions. These insertions were characterized by loss of the 3.6-kb *Bam*HI-Q DNA fragment (within which TK sequences are located; 17, 32) present in the parental MP strain and the appearance of a new 6.5-kb DNA fragment that hybridized both to the TK probe and the insert probe and comigrated with the 6.5-kb fragment in pKZ801. The 7.0-kb HSV-1 DNA fragment, which contains the gene for gC-1 and therefore hybridizes to the insert probe, comigrated with this new 6.5-kb fragment on this gel, partially obscuring the hybridization results with the insert probe.

In similar fashion, Fig. 3 shows that four AraT$^r$ isolates (705-4, 705-5, 705-6, and 701-10) from transfections with either pKZ701 or pKZ705 contain insertions, and Fig. 4 shows that three AraT$^r$ isolates (750-3, 750-4, and 750-5) contain insertions after transfections with pKZ750. Similar results were also obtained in analyses of genomic DNAs from AraT$^r$ viral isolates obtained after transfections with pKZ672 and pKZ669 (data not shown).

The AraT$^r$ isolates shown in Fig. 2, 3, and 4 that do not have insertions of HSV-2 DNA sequences into the TK gene are presumed to be spontaneous tk$^-$ mutants.

**Analysis of AraT$^r$ recombinant viruses for expression of gF.** To determine whether the AraT$^r$ recombinant viruses expressed gF, immunoprecipitation experiments were performed in which [$^{35}$S]methionine-labeled infected cell extracts were reacted with anti-gF-2 (III188) antibody, anti-gF-2 antiserum R#71, or both.

Recombinant viruses that contained the HSV-2 4.9-kb *Sac*I fragment (0.608 to 0.642 map units) inserted in either orientation into their TK genes expressed gF (data not shown). Similarly, recombinant viruses that contained insertions of the HSV-2 2.9-kb *Sal*I DNA fragment (0.621 to 0.641 map units), inserted in either orientation into their TK genes, also expressed gF (Fig. 5). As expected, viruses without insertions (801-4 and 802-3) did not express gF.

Figure 6 shows immunoprecipitations with AraT$^r$ recombinant viruses that contained insertions of the HSV-2 3.1-kb *Sac*I-*Bgl*II DNA fragment (0.608 to 0.630 map units) or the 1.8-kb *Bgl*II-*Sac*I DNA fragment (0.630 to 0.642 map units). Insertion of these DNA fragments did not result in the expression of gF, demonstrating that DNA sequences necessary for gF expression are not wholly contained within either region. Figure 7 summarizes the results obtained from analysis of the recombinant viruses. These results map the gene for gF to a region of the HSV-2 genome (0.62 to 0.64 map units) that is colinear with the gene for gC (0.63 to 0.64 map units; 9, 14) in the HSV-1 genome.

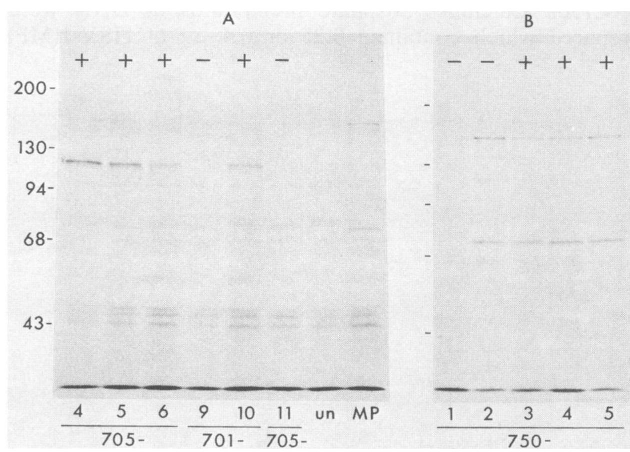


FIG. 6. (A) Immunoprecipitates obtained with anti-gF-2 (R#71) antiserum from extracts of uninfected HEp-2 cells (un) or HEp-2 cells infected with MP or AraT$^r$ viruses. The AraT$^r$ viruses were obtained from transfections with pKZ701 or pKZ705 DNA and MP DNA. Infected cells were labeled with 25 μCi of [$^{35}$S]methionine per ml from 4 to 24 h after infection. Uninfected cells were labeled during this same time period. (B) Immunoprecipitates obtained with a combination of both anti-gF-2 (III188) antibody and anti-gF-2 (R#71) antiserum from extracts of HEp-2 cells infected with AraT$^r$ viruses obtained from transfections with pKZ750 DNA and MP DNA. Infected cells were labeled with 30 μCi of [$^{35}$S]methionine per ml from 4 to 24 h after infection. The + and − symbols present in both A and B show the results of Southern blot analyses performed with the DNA from each of the AraT$^r$ viruses and indicate the presence or absence, respectively, of HSV-2 DNA insertions within the TK genes of the AraT$^r$ viruses.

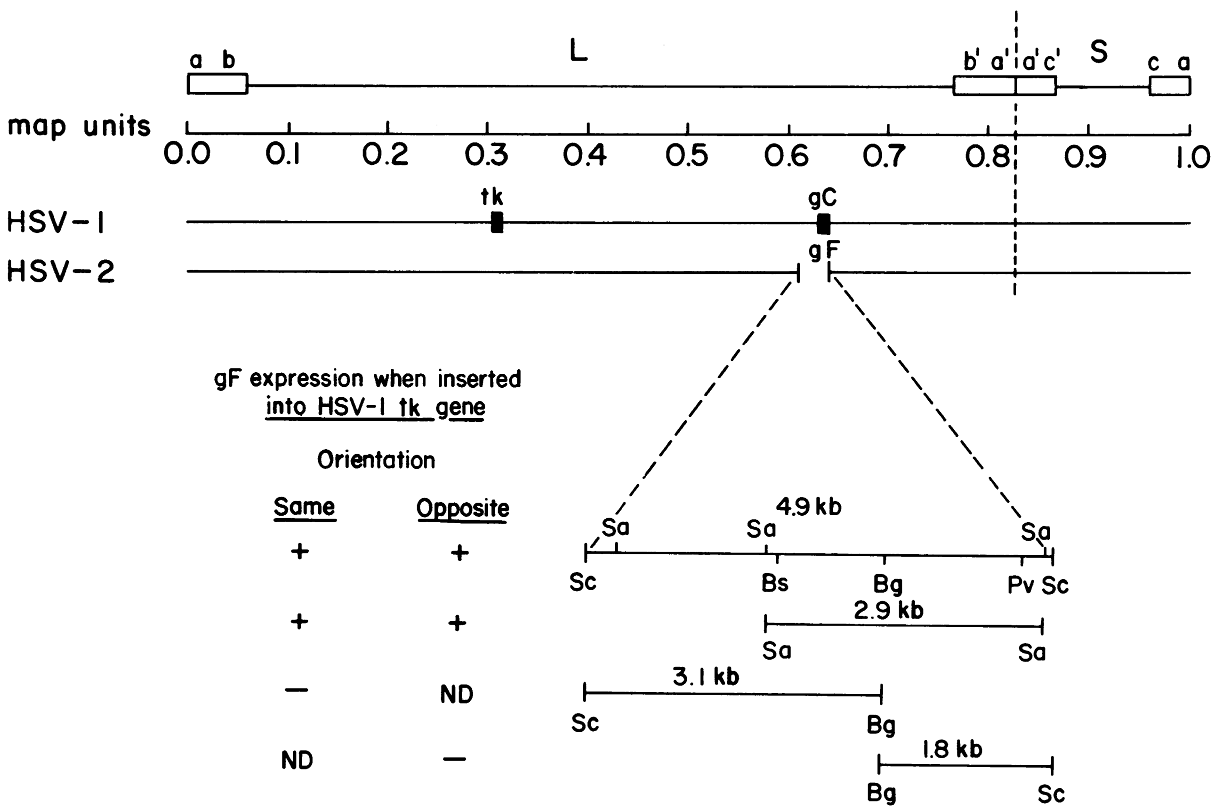


FIG. 7. Location of the gene for gF on the HSV-2 genome and the genes for gC (9, 14) and TK (17, 32) on the HSV-1 genome and a summary of the results obtained from screening AraT$^r$ viruses that contained insertions of HSV-2 DNA sequences in their TK genes for expression of gF. HSV-2 DNA fragments were inserted into the HSV-1 TK gene in the same orientation as they occur in the HSV-2 genome, or in the opposite orientation. The + symbol denotes expression of gF, and the − symbol denotes absence of expression of gF. ND indicates not done. The vertical dashed line marks the boundary between the L and S components of the HSV genome and the white boxes represent the reiterated sequences of the L and S components (27). Abbreviations: Sa, *Sal*I; Sc, *Sac*I; Bs, *Bst*EII; Bg, *Bgl*II; Pv, *Pvu*I.

**Functional correlation between gC-1 and gF-2 by the identification of gF$^-$ mutants of HSV-2.** A *syn* mutant of HSV-2 was examined to determine whether this mutant, in analogy with some HSV-1 *syn* mutants, was gF$^-$. HSV-2(G$_P$) is a spontaneous syncytial variant of HSV-2(G) isolated by Cassai et al. (4). These authors showed that G$_P$ virions contained a glycoprotein of the appropriate electrophoretic mobility to be gF-2, and we found that gF was precipitated from G$_P$-infected cell extracts by anti-gF-2 (III188) antibody (data not shown). However, examination of G$_P$ plaque morphology on both HEp-2 and Vero cells demonstrated two different phenotypes in the viral population: syncytial plaques and smaller plaques in which no fusion was evident, although progeny from these plaques proved to have limited fusion-inducing capacity. Progeny from three syncytial plaques (S1, S2, and S3) and three of the other plaques (NS1, NS2, and NS3) were isolated from Vero cell monolayers and plaque purified three times, and then viral stocks of these isolates were prepared in HEp-2 cells. Immunoprecipitation experiments performed with these six viral isolates showed that NS1, NS2, and NS3 expressed gF, whereas S1, S2, and S3 did not (Fig. 8). Analysis of the DNAs of these six isolates with the restriction enzyme *Bgl*II resulted in fragment patterns similar to that of HSV-2(G) and dissimilar to that of HSV-1 (data not shown), confirming that all six isolates were HSV-2 strains. Also, none of the six isolates expressed gC-1 as assayed by immunoprecipitations with anti-gC-1 (151.2 and II73) antibodies, but all six isolates did express gD as assayed by immunoprecipitations with anti-gD-1/2 (III114) antibody (data not shown). These results indicate that gF-2, like gC-1, is nonessential for viral growth in tissue culture and that expression of gF$^-$ and *syn* phenotypes is coincident in certain HSV-2 *syn* mutants.

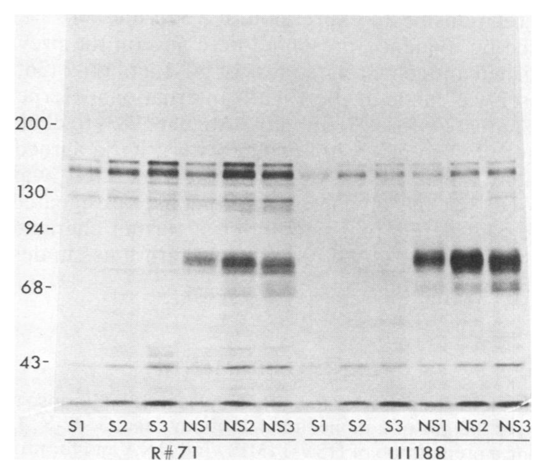


FIG. 8. Polypeptides precipitated by anti-gF-2 (III188) antibody or anti-gF-2 antiserum R#71 from extracts of HEp-2 cells infected with HSV-2(G$_P$) isolates S1, S2, S3, NS1, NS2 or NS3. The infected cells were labeled with 20 μCi of [$^{35}$S]methionine per ml from 4 to 24 h after infection.

## DISCUSSION

The results presented here, together with other evidence summarized above (22, 36, 37), demonstrate that gF is the HSV-2 counterpart of HSV-1 gC.

The gene for gF maps within a 2.9-kb *Sal*I DNA fragment (approximately 0.62 to 0.64 map units). This HSV-2 region shares DNA homology with the HSV-1 *Sal*I-R DNA fragment, within which the gC gene is located (9, 14), and the gC gene (approximately 0.63 to 0.64 map units) is colinear with the gene for gF. Because gF is expressed upon insertion of the 2.9-kb *Sal*I DNA fragment, in either orientation, into the HSV-1 TK gene, both coding and *cis*-acting regulatory DNA sequences necessary for expression of gF in infected cells are probably located within this DNA fragment. Analysis of viruses containing *Sac*I-*Bgl*II HSV-2 DNA fragments inserted into the TK gene demonstrated that sequences both to the left and right of the *Bgl*II site (0.630 map units) in the 2.9-kb *Sal*I DNA fragment are necessary for the expression of gF.

DNA sequence determinations of the HSV-1 and HSV-2 genomes in this region (9; R. Frink, K. Draper, M. Swain, D. Galloway, and E. Wagner, manuscript in preparation) have shown that, based on the best sequence fit, this *Bgl*II site lines up with the HSV-1 genome 35 base pairs to the right of the *Eco*RI site, which is located within the HSV-1 gC structural gene approximately 450 base pairs downstream of the putative initiation codon. Also, an mRNA species that has been located in this region of the HSV-2 genome is the same size as the mRNA species encoding HSV-1 gC (approximately 2.5 kb; Frink et al., in preparation), suggesting that gF may be translated from this mRNA. Comparisons of the complete nucleotide sequences of the genes for gF and gC are of particular importance and interest because gF and gC exhibit differences in size and antigenicity (1, 2, 22, 36, 37) that suggest more divergence between these HSV-1 and HSV-2 genes than is evident for other glycoprotein genes.

Comparison of the mapping data presented here with the data of Marsden et al. (16) suggests that the 63K HSV-2 glycoprotein they mapped to the region from 0.57 to 0.66 map units is gF.

Identification of an HSV-2 $gF^-$ mutant, in association with a *syn* phenotype, demonstrates not only that gF is analogous to gC in being nonessential for viral growth in cell culture, but also that it may share the same, as yet undefined, relationship to expression of a *syn* marker.

Based on evidence presented here and on the previously established antigenic relatedness of gC-1 and gF-2 (36, 37), a proposal was made at the Eighth International Herpesvirus Workshop at Oxford (31 July to 5 August 1983) to change the name of gF-2 to gC-2, in keeping with criteria agreed upon for the naming of HSV glycoproteins (5). It was also proposed that the glycoprotein previously designated gC-2 (28) be considered unnamed until it is further characterized, and that the name gF be retired from future use in designating HSV glycoproteins.

## ACKNOWLEDGMENTS

We thank B. Roizman for the gift of a plasmid, J. Glorioso and M. Levine for the gift of a monoclonal antibody, and G. T.-Y. Lee for providing a preparation of HSV-1 (MP) viral DNA and for providing DNA from pTYL302 (pTYL302 contains HSV-2 [333] DNA between the *BamHI* sites at 0.607 and 0.690 map units), which was used in the construction of pKZ672 and pKZ669. We also thank Richard Aspell and Mary Lynn Parish for excellent technical assistance and E. K. Wagner and D. Galloway for communicating results before their publication.

This work was supported by grants from the American Cancer Society and Public Health Service grants CA21776 and CA19264 from the National Cancer Institute (to P.G.S.). K.M.Z. was a trainee of the National Service Research Award 5 T32 GM07197.

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